Talanta 81 (2010) 222–227

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A rapid and sensitive alcohol oxidase/catalase conductometric biosensor for alcohol determination

M. Hnaien, F. Lagarde*, N. Jaffrezic-Renault

Université de Lyon, Lyon1, Laboratoire des Sciences Analytiques, CNRS UMR 5180, Bâtiment CPE, 43, boulevard du 11 novembre 1918, 69622 Villeurbanne Cedex, France

ARTICLE INFO

Article history:
Received 8 October 2009
Received in revised form
24 November 2009
Accepted 29 November 2009
Available online 3 December 2009

Keywords:
Alcohol oxidase
Catalase
Poly(vinyl alcohol)
Conductometric biosensor
Alcohol determination
Interdigitated microelectrodes

ABSTRACT

A new conductometric biosensor has been developed for the determination of short chain primary aliphatic alcohols. The biosensor assembly was prepared through immobilization of alcohol oxidase from *Hansenula* sp. and bovine liver catalase in a photoreticulated poly(vinyl alcohol) membrane at the surface of interdigitated microelectrodes. The local conductivity increased rapidly after alcohol addition, reaching steady-state within 10 min. The sensitivity was maximal for methanol ($0.394 \pm 0.004\ \mu S\ \mu M^{-1}$, $n = 5$) and decreased by increasing the alcohol chain length. The response was linear up to 75 μM for methanol, 70 μM for ethanol and 65 μM for 1-propanol and limits of detection were 0.5 μM, 1 μM and 3 μM, respectively (S/N = 3). No significant loss of the enzyme activities was observed after 3 months of storage at 4 °C in a 20 mM phosphate buffer solution pH 7.2 (two or three measurements per week). After 4 months, 95% of the initial signal still remained. The biosensor response to ethanol was not significantly affected by acetic, lactic, ascorbic, malic, oxalic, citric, tartaric acids or glucose. The bi-enzymatic sensor was successfully applied to the determination of ethanol in different alcoholic beverages.

1. Introduction

Alcohol consumption or inhalation can seriously affect human health and, in the most extreme cases, cause death [1]. In cosmetics, ethanol is traditionally used as a solvent or disinfectant but adverse effects on skin have been also observed [2]. Quantitative determination of alcohols is thus important in clinical analysis. Accurate quantitation of ethanol concentration is also crucial to the design, assessment and improvement of fermentation processes, and to control the quality of food and alcoholic beverages such as beer, wine, liquor and spirits. Many analytical methods have been developed for the determination of ethanol and other aliphatic alcohols, such as methanol. These include chemical methods, e.g. colorimetry, refractometry, chromatographic and spectroscopic techniques, which are time consuming and, for the most advanced of them, require expensive instrumentation and trained operators [3–5]. Such disadvantages can be overcome by the use of biosensors, which are very attractive alternatives for the simple, rapid and possible on-line detection of many pollutants. Whole cells [6–9] or enzymes [3,10–28] have been used for the preparation of alcohol biosensors. Enzymes are one of the essential components of living systems, catalyzing with a relatively high specificity almost all chemical transformations that occur during cell metabolism.

Alcohol enzyme biosensors described in the literature are mainly based on alcohol dehydrogenase (ADH) [10–16] and alcohol oxidase (AOX) [3,17–27], more rarely on catalase [28]. This latter needs hydrogen peroxide as co-substrate. ADH catalyzes the reversible oxidation of primary aliphatic and aromatic alcohols other than methanol but requires the presence of nicotinamide adenine dinucleotide (NAD^+) [10–15] or pyrroloquinoline quinone (PQQ) [16]. PQQ-dependent alcohol dehydrogenase (PQQ-ADH) exhibits better selectivity to ethanol than NAD-ADH or AOX but is not commercially available in the purified form, and purified PQQ enzymes have a low stability [9]. Ethanol biosensors using AOX as biorecognition element are consequently the most abundant and have been recently reviewed [3]. AOX is an oligomeric enzyme responsible for the oxidation of low molecular weight primary alcohols, using molecular oxygen (O_2) as the electron acceptor and producing acetaldehyde and hydrogen peroxide according to Eq. (1). Due to the strong oxidizing character of O_2 the oxidation of alcohols by AOX is irreversible:

$$RCH_2OH + O_2 \xrightarrow{AOX} RCHO + H_2O_2 \quad (1)$$

The reaction may be followed by measuring either the decrease of O_2 or the increase of H_2O_2 concentration using optical or electrochemical detections. Until now, all the electrochemical transducers proposed are amperometric or voltametric.

In this work, we propose an original biosensor for alcohol determination, prepared by co-immobilizing AOX and catalase on the surface of interdigitated thin-film electrodes in view of a con-

* Corresponding author. Tel.: +33 472432627; fax: +33 472448319.
E-mail address: florence.lagarde@univ-lyon1.fr (F. Lagarde).

0039-9140/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2009.11.061

ductometric detection. This mode of transduction offers several advantages: (i) thin-film electrodes are suitable for miniaturization and large scale production using inexpensive technology, (ii) it does not require any reference electrode and differential mode measurements allow cancellation of many interferences, (iii) it is not light sensitive, (iv) the driving voltage can be sufficiently low to decrease significantly the power consumption, and (v) large spectrum of compounds of different nature can be determined on the basis of various reactions and mechanisms [29]. In addition, this is the first time that a bi-enzymatic biosensor based on AOX/catalase has been proposed. Catalase is a common enzyme found in nearly all living organisms, where it catalyzes both the decomposition of hydrogen peroxide into water and oxygen and the degradation of ethanol in the presence of H_2O_2 according to reactions (2) and (3):

$$2H_2O_2 \xrightarrow{\text{Catalase}} O_2 + 2H_2O \quad (2)$$

$$CH_3CH_2OH + H_2O_2 \xrightarrow{\text{Catalase}} CH_3CHO + 2H_2O \quad (3)$$

The first advantage of the bi-enzymatic system proposed is that ethanol acts as a substrate for both enzymes. In addition, hydrogen peroxide, produced by the alcohol oxidation at the outer membrane of the biosensor (alcohol oxidase membrane), is used as a co-substrate in the inner membrane containing catalase and regenerates oxygen, required for reaction (1). In addition, H_2O_2 consumption by catalase can help to improve AOX stability [26].

2. Experimental

2.1. Chemicals

Poly(vinyl alcohol) bearing photopolymerizable styrylpyridinium groups (PVA-SbQ) was purchased from Toyo Gosei (Shiba, Japan). Alcohol oxidase (AOX, EC 1.1.3.13, 7.7 U mg^{-1} solid, from methylotrophic yeast *Hansenula* sp.), catalase (EC1.11.1.6, 2950 U mg^{-1} solid, from bovine liver), bovine serum albumin (BSA), glutaraldehyde (GA) (grade II, 25% aqueous solution), D-glucose (>99.5%), NaH_2PO_4 (>99%) and $Na_2HPO_4{\cdot}12H_2O$ (>99%) were obtained from Sigma–Aldrich. Methanol, ethanol and 1-propanol (>99.8%) and citric acid (>99.5%) were from Fluka, L-lactic acid (90% solution in water), DL-malic acid (>99%) and glycerol (>99%) from Acros Organics, L-ascorbic acid (>99.7%) from Merck, oxalic acid (98%) from Lancaster and L-tartaric acid (>99.5%) from Carlo Erba. The reagents were used as purchased. Stock concentrated solutions were prepared in 5 mM phosphate buffer solution (pH 7.2) and stored at 4 °C. All aqueous solutions were prepared using 18 MΩ cm ultrapure water obtained from an Elgastat UHQ II purification system (Elga Labwater, Le Plessis Robinson, France).

2.2. Sensor design

The conductometric transducers (Fig. 1), consisting of two identical pairs of gold interdigitated thin-film electrodes (thickness: 150 nm), were fabricated by vacuum deposition on a ceramic substrate (5 mm × 30 mm) at the Lashkaryov Institute of Semiconductor Physics (Kiev, Ukraine). A 50-nm thick intermediate chromium layer was used for better gold adhesion. The dimension of each interdigital space and digit was 20 μm and the length of the digits was about 1.0 mm. The sensitive area of each pair of electrodes was about 1 mm^2 [29].

2.3. Preparation of the biosensor

A three-step procedure was followed to prepare the bi-enzymatic biosensor. As a differential experimental setup was used, 0.2 μL of a 20 mM phosphate buffer pH 7.2 containing 6% (m/v) BSA, 10% (m/v) glycerol and 4% of catalase was first deposited on the working pair of electrodes, while on the reference electrodes only a mixture containing 10% (m/v) BSA and 10% (m/v) glycerol was applied. Then the sensor chip was allowed to dry for about 15 min at room temperature. A second layer was then deposited following the same protocol except that catalase was replaced by alcohol oxidase in the enzymatic solution. In the last step of the procedure (immobilization of enzymes), both pairs of electrodes were covered by 0.2 μL of a 10% (m/v) PVA-SbQ aqueous solution. PVA cross-linking was performed by irradiating the microelectrodes for 20 min at 365 nm in a Bio-link BLX UV-cross-linker (Vilbert Lourmat) equipped with five 8 W lamps. A schematic representation of the final biosensor architecture is given in Fig. 2.

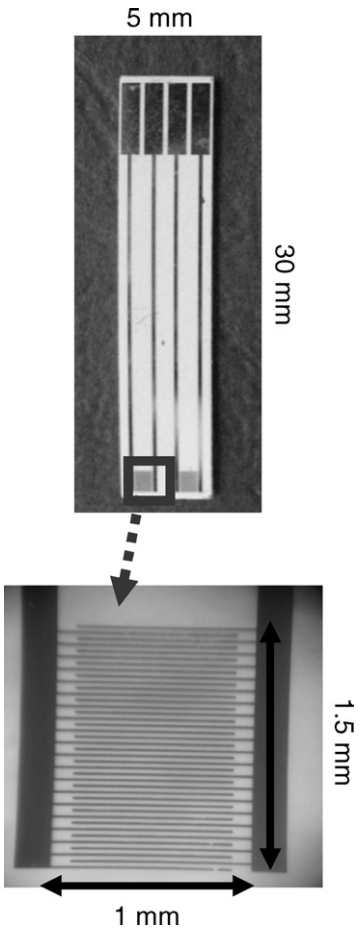

Fig. 1. General view of the conductometric transducer and its interdigitated microelectrodes.

Biosensors were used just after preparation or stored at 4 °C in a 20 mM phosphate buffer solution, pH 7.2 until measurements.

2.4. Conductometric measurements

Microelectrodes were placed in a glass cell filled with 5 mL of a 5 mM phosphate buffer pH 7.2. The solution was stirred vigorously. Measurements were then performed at 23 ± 2 °C by applying to the differential pairs of electrodes an alternating voltage (10 mV amplitude, 100 kHz frequency) generated by a low-frequency wave-form generator (SR830 Lock-in amplifier from Stanford Research Systems). These conditions allowed to reduce faradaic processes, double-layer charging and concentration polarization at the microelectrode surface. After stabilization of the differential output signal, small aliquots (5–80 μL) of a concentrated substrate solu-

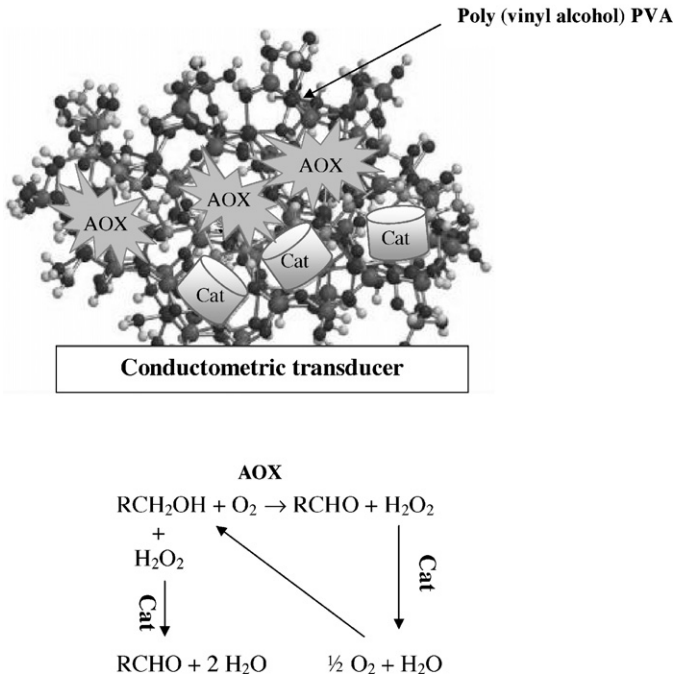

Fig. 2. Schematic representation of the bi-enzyme sensor architecture and working principle.

tion were added in order to achieve final concentrations between 10 μM and 150 μM.

3. Results and discussion

3.1. Analytical characteristics of the catalase/AOX biosensor

3.1.1. Response time

Fig. 3 shows a typical biosensor response obtained after ethanol addition. The injection of ethanol into the measurement cell causes a rapid and significant increase of the conductivity due to the enzymatic oxidation of ethanol and H_2O_2. Equilibrium between the production of ions due to the enzymatic reaction inside the membrane and the influx of ions into the membrane via a mediated transport mechanism, with buffer species acting as a carrier, was achieved within 10 min. The steady-state response time, defined as

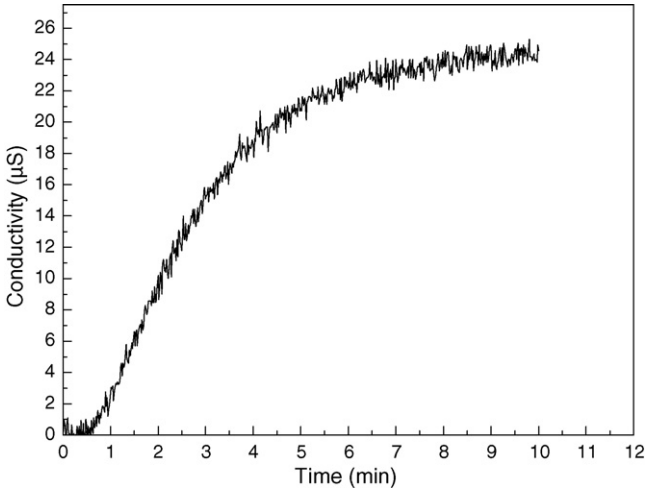

Fig. 3. Typical response of the conductometric biosensor. Ethanol concentration: 68 μM; pH 7.5; temperature 23 ± 2 °C.

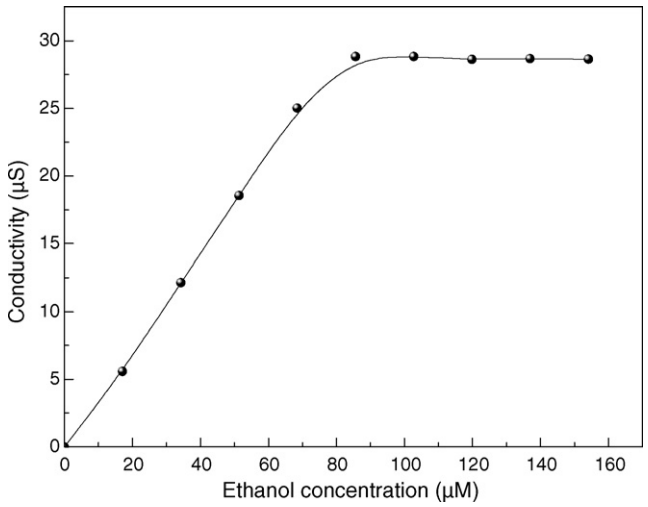

Fig. 4. Evolution of the biosensor response with ethanol concentration (n = 5). Measurements in 5 mM phosphate buffer pH 7.5; temperature 23 ± 2 °C.

the time required to reach 90% of the steady-state signal [30], was close to 5 min. The same trends and similar response times were obtained following the injection of two other short chain alcohols, methanol and 1-propanol (data not shown).

3.1.2. Linear range and limit of detection

The relationship between biosensor response and ethanol concentration was examined in the 0–150 μM range. For that, nine standard solutions were used and five measurements were performed at each concentration level. Results are presented in Fig. 4. A lack of fit test at the 5% level proved that the biosensor response is linear up to 70 μM ethanol. The correlation coefficient and the sensitivity were 0.9983 and 0.363 ± 0.005 μS μM^{-1}, respectively, and the intercept was not significantly different from zero. Saturation of the biosensor was achieved at a very low concentration level, when compared to that of the other AOX-based biosensors already reported (Table 1). This result may be due to the fact that lower enzyme amounts are deposited on the working electrode. In addition, immobilization of AOX in PVA can modify its conformation and decrease the accessibility of binding sites. As a consequence, concentrated samples will have to be properly diluted to fall within this narrow concentration range. The limit of detection (LOD) was 1 μM (S/N = 3), which is much lower than the values reported for most of the other AOX-based biosensors.

The same methodology was applied to methanol and 1-propanol. The decrease of the chain length improved the linear domain and the sensitivity of the biosensor, yielding the lowest LOD for methanol (0.5 μM) and the highest for 1-propanol (3 μM), as shown in Table 1.

Similar results have been already reported for other AOX-based biosensors [17–19,21,22,24,26] and can be attributed to an increase of AOX affinity for shorter chain primary alcohols. Apparent Michaelis–Menten constants of free AOX, as determined by Yildiz and Toppare [31], are 0.5, 1.5 and 9.7 mM for methanol, ethanol and 1-propanol, respectively.

3.1.3. Short-term reproducibility

The short-term reproducibility of the biosensor response was tested on three different sensors at four concentration levels in the 30–70 μM range. The variation coefficient obtained from five measurements performed within 1 day with the same sensor was very good as it was between 1.5% and 4% in the concentration range studied.

Table 1
Analytical characteristics of some AOX and catalase based alcohol biosensors.

Enzyme(s)	Transducer	Substrate	Sensitivity	Linearity	Detection limit	Real samples	Stability	References
AOX/catalase	Conductometric	Methanol	$0.394 \pm 0.004\ \mu S\ \mu M^{-1}$	Up to 75 μM	0.5 μM	White wine, pineau, pastis	No loss after 12 weeks. Only 5% signal decrease after 16 weeks (two to three measurements per week). Storage in buffer at 4 °C	This work
		Ethanol	$0.363 \pm 0.005\ \mu S\ \mu M^{-1}$	Up to 70 μM	l μM			
		1-Propanol	$0.317 \pm 0.006\ \mu S\ \mu M^{-1}$	Up to 65 μM	3 μM			
AOX	Amperometric	Methanol	$\sim 230\ mA\ M^{-1}\ cm^{-2}$	Up to 0.1 mM	0.1 μM	Wines, sakes, beers, soy sources	1 week (10 measurements a day). 50% of sensitivity lost after 2 weeks. Storage in buffer at 4 °C	[17]
		Ethanol	$\sim 40\ mA\ M^{-1}\ cm^{-2}$	Up to 0.4 mM	0.5 μM			
AOX	Amperometric	Methanol	$\sim 1.2\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.	Red and white wines	45% of sensitivity lost after 8 days (one measurement a day). Storage in buffer at 4 °C	[18]
		Ethanol	$0.97\ mA\ M^{-1}\ cm^{-2}$	Up to 3 mM	10 μM			
		1-Propanol	$<0.05\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.			
		1-Butanol	$<0.05\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.			
AOX	Amperometric	Methanol	$9.1\ mA\ M^{-1}\ cm^{-2}$	Up to 3.7 mM	n.r.	n.r.	A few days (94% of sensitivity lost after 36 days (132 measurements))	[19]
		Ethanol	$3.4\ mA\ M^{-1}\ cm^{-2}$	Up to 3 mM	n.r.			
		n-Butanol	$0.6\ mA\ M^{-1}\ cm^{-2}$	Up to 6.2 mM	n.r.			
		Benzyl alcohol	$0.4\ mA\ M^{-1}\ cm^{-2}$	Up to 5.2 mM	n.r.			
AOX	Amperometric	Ethanol	n.r.	n.r.	3.5×10^{-2} (% v/v)	Red and white wines	Decrease by 4% a day at continuous work during 5 days	[20]
AOX	Amperometric	Methanol	$52\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.	Red wines	No loss after 90 injections carried out in 3 h; 5% of sensitivity lost after 6 h of on-line continuous monitoring	[21]
		Ethanol	$4.1\ mA\ M^{-1}\ cm^{-2}$	Up to 0.75 mM	2.3 μM			
		n-Propanol	$3.2\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.			
		n-Butanol	$1.2\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.			
AOX	Amperometric	Methanol	$0.72\ mA\ M^{-1}\ cm^{-2}$	n.r.	22 μM	Red and white wines	12% of sensitivity lost after 6 weeks Storage in buffer at 4 °C	[22]
		Ethanol	$0.70\ mA\ M^{-1}\ cm^{-2}$	0–0.6 mM	25 μM			
		1-Propanol	$0.27\ mA\ M^{-1}\ cm^{-2}$	n.r.	53 μM			
AOX	Amperometric	Ethanol	n.r.	0.3–20 mM	300 μM	Red, white and rose wines, must	No loss after 2 months, ~90% after 3 months (several measurements a week). Dry storage at 4 °C	[23]
AOX/HRP	Amperometric	Methanol	$5\ mA\ M^{-1}\ cm^{-2}$	Up to 0.4 mM	8.5 μM	Beers, red and white wines, liquors	No loss after 15 days (three measurements a day). Dry storage at 4 °C	[24]
		Ethanol	$1.9\ mA\ M^{-1}\ cm^{-2}$	Up to 4 mM	19 μM			
AOX/HRP	Amperometric	Ethanol	$0.82–1.4\ mA\ M^{-1}\ cm^{-2}$	n.r.	n.r.	Red and white wines	n.r.	[25]
AOX/HRP	Amperometric	Methanol	$6.6\ mA\ M^{-1}\ cm^{-2}$	Up to 1 mM	n.r.	Red and white wines	50% current drop after about 14 days. Storage in buffer at 4 °C	[26]
		Ethanol	$2.7\ mA\ M^{-1}\ cm^{-2}$	Up to 2 mM	n.r.			
		n-Propanol	n.r.	Up to 4 mM	n.r.			
		n-Butanol	n.r.	Up to 8 mM	n.r.			
AOX/HRP	Amperometric	Ethanol	$26.7\ mA\ M^{-1}\ cm^{-2}$	Up to 4 mM	10	n.r.	15% of sensitivity lost after 6 days (one measurement a day). Storage in buffer at 4 °C	[27]
Catalase	Amperometric	Ethanol	n.r.	Up to 1 mM	50 μM	Beers	No loss after 2 weeks. 22% of activity lost after 6 weeks (one measurement a week); storage in water at 4 °C	[28]

n.r.: not reported; HRP: horseradish peroxidase.

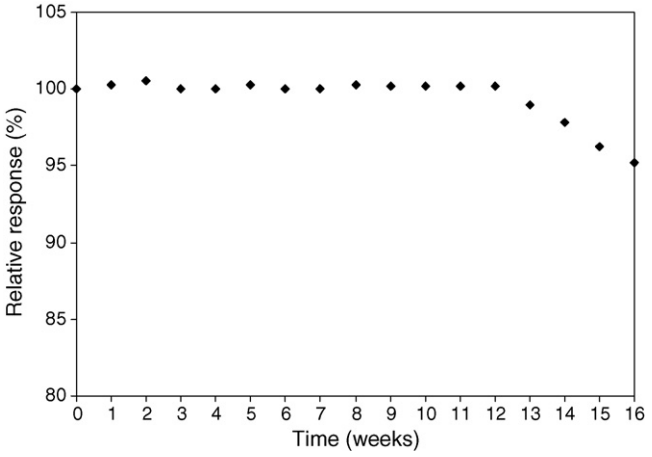

Fig. 5. Study of the biosensor long-term storage stability. The electrodes were stored at 4 °C in 20 mM phosphate buffer pH 7.2 between two determinations. Measurements were performed at 23 ± 2 °C in 5 mM phosphate buffer pH 7.2 for 50 μM ethanol.

3.1.4. Stability

Long-term storage stability is one of the key factors of a sensor performance. One sensor was fabricated following the protocol described in Section 2 and its response was measured two to three times a week over a 4 months period. Meanwhile, the biosensor was stored at +4 °C in 20 mM phosphate buffer (pH 7.2). As seen in Fig. 5, the biosensor was stable over a very long period. The signal remained stable for 3 months. A decrease of the response was further observed, but only 5% of the initial value was lost after 4 months. This excellent storage stability largely exceeds those reported before (Table 1), showing that PVA coating was efficient to protect enzymes from external aggressions. The slight decrease observed after 4 months of storage may be attributed either to a progressive decrease of enzymatic activity or to the degradation of the PVA membrane, resulting in enzyme release.

3.2. Determination of ethanol in natural samples

The biosensor was used for the determination of ethanol in alcoholic beverages. Prior to this, an interference study was carried out. Several substances that may be present in this kind of sample, e.g. lactic, ascorbic, malic, oxalic, citric, tartaric acids and glucose, were tested. The usual concentration of methanol is much lower than ethanol content, and then no significant interference is expected to occur with this compound. The study was performed at a 50 μM ethanol concentration for a 1:1 ethanol/interferent molar ratio. As seen in Fig. 6, the biosensor response was not significantly affected.

Three French alcoholic beverages (Pineau, Ricard pastis and Beaumes de Venise white wine) were then analyzed. Taking into account that the concentration of ethanol in that kind of sample is far outside the working range of the sensor (from 15% to 45%, representing 2.6–7.1 M ethanol), a 100-fold dilution of the beverages was necessary. This latter was performed in a 5 mM phosphate buffer solution pH 7.2 and only 5 μL of the diluted samples were subsequently injected in the measurement cell. This considerable dilution offers the advantage to minimize matrix effects but may be carefully carried out using accurate instrumentation in order to minimize the dilution errors. Table 2 shows that ethanol contents determined using the catalase–AOX biosensor developed in this work are in good agreement with those declared by the producer.

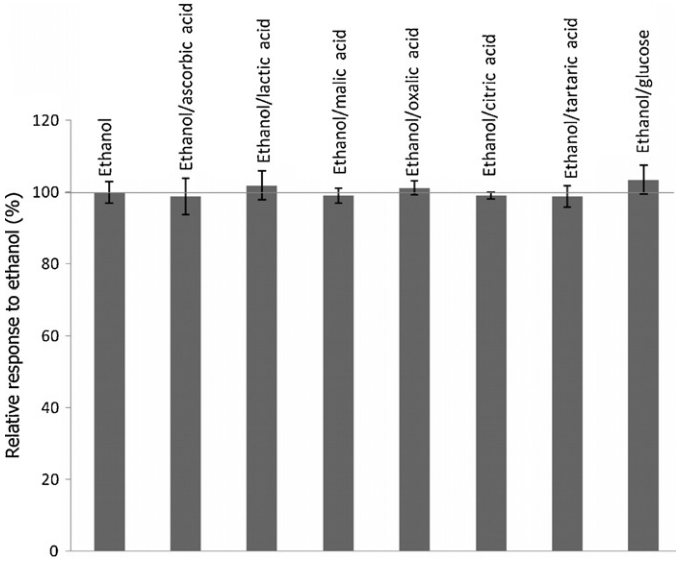

Fig. 6. Comparison of the ethanol biosensor response ($n = 3$) in the absence and in the presence of possible interferents (1:1 ethanol:interferent molar ratio). Measurements were performed at 23 ± 2 °C in 5 mM phosphate buffer pH 7.2 for 50 μM ethanol.

Table 2
Ethanol determination in alcoholic beverages.

Alcohol content (% v/v)	Pineau	Ricard pastis	Beaumes de Venise
Declared by the manufacturer	17	45	15.5
Found	17.3 ± 0.3	45.5 ± 0.1	16 ± 0.2

4. Conclusion

A rapid, sensitive and very stable conductometric biosensor based on AOX and catalase enzymes was developed for alcohol determination. The biosensor assay requires only the dilution of the samples and takes a very short time compared to conventional procedures. A sensitivity of 0.363 μS μM^{-1} and a detection limit of 1 μM were obtained for ethanol. The response was linear up to 70 μM. The biosensor was successfully applied to the quantification of ethanol in three French alcoholic beverages (Pineau, Ricard pastis, Beaumes de Venise white wine), demonstrating the great potential of the proposed alcohol biosensor for practical applications in food analysis.

Acknowledgement

This work was supported by the Direction Générale de l'Armement (DGA), which is gratefully acknowledged.

References

[1] T. Zima, L. Fialov, O. Mestek, M. Janebov, J. Crkovska, I. Malbohan, S. Stipek, L. Mikulfkova, P. Popov, J. Biomed. Sci. 8 (2001) 59–70.
[2] M.G. Neuman, J.A. Haber, I.M. Malkiewicz, R.G. Cameron, G.G. Katz, N.H. Shear, Alcohol 26 (2002) 179–190.
[3] A.M. Azevedo, D.M.F. Prazeres, J.M.S. Cabral, L.P. Fonseca, Biosens. Bioelectron. 21 (2005) 235–247.
[4] B.S. De Martinis, M.A. Martins Ruzzene, C.C. Santos Martin, Anal. Chim. Acta 522 (2004) 163–168.
[5] H. Li, X.-S. Chaia, Y. Deng, H. Zhana, S. Fua, J. Chromatogr. A 1216 (2009) 169–172.
[6] L. Rotariu, C. Bala, V. Magearu, Anal. Chim. Acta 513 (2004) 119–123.
[7] E. Akyilmaz, E. Dinçkaya, Biosens. Bioelectron. 20 (2005) 1263–1269.
[8] E.A. Voronova, P.V. Iliasov, A.N. Reshetilov, Anal. Lett. 41 (2008) 377–391.
[9] M. Valach, J. Katrlik, E. Sturdik, P. Gemeiner, Sens. Actuators B 138 (2009) 581–586.

[10] K. Svensson, L. Bülow, D. Kriz, M. Krook, Biosens. Bioelectron. 21 (2005) 705–711.
[11] C.R. Raj, S. Behera, Biosens. Bioelectron. 21 (2005) 949–956.
[12] R. Antiochia, I. Lavagnini, Anal. Lett. 39 (2006) 1643–1655.
[13] H.N. Choi, Y.-K. Lyu, J.H. Han, W.-Y. Lee, Electroanalysis 19 (2007) 1524–1530.
[14] B. Bucur, G.L. Radu, C.N. Toader, Eur. Food Res. Technol. 226 (2008) 1335–1342.
[15] C.A. Lee, Y.-C. Tsai, Sens. Actuators B 138 (2009) 518–523.
[16] J. Razumiene, V. Gureviciene, V. Laurinavicius, J.V. Grazulevicius, Sens. Actuators B 78 (2001) 243–248.
[17] F. Mizutani, S. Yaruki, S. Iijiama, Anal. Sci. 13 (1997) 83–87.
[18] N.G. Patel, S. Meier, K. Cammann, G.C. Chemnitius, Sens. Actuators B 75 (2001) 101–110.
[19] H. Gülce, A. Gülce, M. Kavanoz, H. Coşkun, A. Yildiz, Biosens. Bioelectron. 17 (2002) 517–521.
[20] L.V. Shkotova, A.P. Soldatkin, M.V. Gonchar, W. Schuhmann, S.V. Dzyadevych, Mater. Sci. Eng. C 26 (2006) 411–414.
[21] D. Carelli, D. Centonze, A. De Giglio, M. Quinto, P.G. Zambonin, Anal. Chim. Acta 565 (2006) 27–35.
[22] M.M. Barsan, C.M.A. Brett, Talanta 74 (2008) 1505–1510.
[23] T.B. Goriushkina, A.P. Soldatkin, S.V. Dzydevych, J. Agric. Food Chem. 57 (2009) 6528–6535.
[24] A. Guzman-Vasquez de Prada, N. Pena, A.J. Mena, A.J. Reviejo, J.M. Pingarrón, Biosens. Bioelectron. 18 (2003) 1279–1288.
[25] I.S. Alpeeva, A. Vilkanauskyte, B. Ngounou, E. Csöregi, I.Y. Sakharov, M. Gonchar, W. Schuhmann, Microchim. Acta 152 (2005) 21–27.
[26] O. Smutok, B. Ngounou, H. Pavlishko, G. Gayda, M. Gonchar, W. Schuhmann, Sens. Actuators B 113 (2006) 590–598.
[27] A.R. Vijayakumar, E. Csöregi, A. Heller, L. Gorton, Anal. Chim. Acta 327 (1996) 223–234.
[28] E. Akyilmaz, E. Dinçkaya, Talanta 61 (2003) 113–118.
[29] N. Jaffrezic-Renault, S.V. Dzyadevych, Sensors 8 (2008) 2569–2588.
[30] D.R. Thevenot, K. Toth, R.A. Durst, G.S. Wilson, Biosens. Bioelectron. 16 (2001) 121–131.
[31] H.B. Yildiz, L. Toppare, Biosens. Bioelectron. 21 (2006) 2306–2310.